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ACETYLCHOLINESTERASE-BASED ELECTROCHEMICAL MULTIPHASE MICROREACTOR FOR DETECTION OF ORGANOPHOSPHOROUS COMPOUNDS (Preprint)

Chelsea N. Monty, Ilwhan Oh, and Richard I. Masel

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14. ABSTRACT
A dual microchannel device with a gas-liquid interface was developed for use as an amperometric biosensor for the detection of organophosphorus compounds based on acetylcholinesterase inhibition. Electric eel acetylcholinesterase was immobilized on the liquid microchannel by creating a cross-linked gel with glutaraldehyde. The system was tested with malathion, an organophosphorus pesticide. The detection limit of the sensor in the parts-per-trillion range and the detection is rapid, sensitive, and selective to only phosphonates. Incorporation of existing acetylcholinesterase biochemistry into a micro-scale sensor also allows the device to be easily portable.

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Electrochemical Gas Sensor, Multiphase Microreactor, gas-liquid interface, acetylcholinesterase, electrochemical sensor

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Acetylcholinesterase-based Electrochemical Multiphase Microreactor for Detection

of Organophosphorous Compounds

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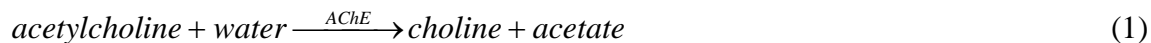
I. Introduction

The demand for sensors to detect harmful materials such as toxic vapors[1,2], explosives[3], and toxic industrial chemicals has increased over the last few years. Thus, there is a need for a hazardous material sensor that has the following properties[4,5]:

- Vapor detection – the target detection molecules are all in the gas phase

- Sensitivity – the vapor phase detection limit must be under a part-per-billion

- Selectivity – the sensor must be reliable and have a low false positive rate

- Portability – a person should be able to easily carry the sensor

Current methods for detection of organophosphates are gas chromatography/mass spectroscopy (GC/MS) and ion mobility spectrometry (IMS)[6]. These detection methods, however, have several key drawbacks. GC/MS is not suited for portable applications and is often very expensive. While, IMS is a faster, less expensive alternative, it is not selective and has a high false positive rate. A microchemical acetylcholinesterase-based (AChE) biosensor offers a promising alternative due to the fast response, high sensitivity, and increased portability of a microchemical sensor and the increased selectivity of an AChE-based biosensor.

In the human body, the primary function of acetylcholinesterase is the hydrolysis of acetylcholine: the principal step that terminates intercellular communication pathways. The hydrolysis of acetylcholine is shown in Equation 1[7,8]. Organophosphates inhibit this hydrolysis by irreversibly binding to the active site of AChE[7,8].

$$acetylcholine + water \xrightarrow{\quad AChE \quad} choline + acetate \qquad\qquad (1)$$

Electrochemical detection of organophosphates is performed using a derivative of acetylcholine, acetylthiocholine, as shown in Equation 2[9,10,11,12,13,14].

$$acetylthiocholine + water \xrightarrow{AChE} thiocholine + acetate \tag{2}$$

The thiocholine product is then oxidized on the electrode surface at 400 mV vs Ag/AgCl. When Equation 2 is inhibited, the production of thiocholine is decreased and a decrease in current is found.

Conventional AChE-based biosensors, reported by previous authors, are used for detection of organophosphorus pesticides in water [(8-35)]. With vapor phase target molecules, we are interested in incorporating the existing, liquid AChE sensor chemistry into a multiphase microreactor. The multiphase microreactor will play a critical role in combining microsensor technology with analytical biochemistry and increase reaction time, sensitivity and selectivity.

A multiphase microchemical system contains reactions, usually occurring at an interface of two or more phases (gas, liquid, solid)[15]. The design and fabrication of the gas-liquid interface is especially difficult because, unlike solid-gas or solid-liquid interfaces, it is fluidic and hard to control. There are two main categories for fabrication of microchannel gas-liquid interfaces: 1) gas-liquid segmented flow[16,17,18,19,20,21,22] 2) gas-liquid parallel flow[23,24,25,26]. Previous authors have achieved gas-liquid segmented flow by combining the two in a hydrophobic microchannel. In order to create gas-liquid parallel flow, the channel walls must be functionalized into hydrophilic and hydrophobic

regions. The liquid then flows near the hydrophilic walls, while the gas stays in the

hydrophobic regions.

The objectives of this paper are 1) to develop a microreactor with a micro-scale gas-

liquid interface, 2) to adapt AChE biochemistry into the microreactor in order to develop

an electrochemical biosensor for the highly selective detection of vapor-phase

organophosphates at below parts-per-billion detection limits.

II. Experimental Methods

A. Fabrication of Microchannel Sensor

The assembly of the microchannel sensor involves three steps: fabrication of

microchannels, deposition of the electrode onto a nanoporous membrane, assembly of

two finished microchannels and nanoporous membrane. A schematic diagram and

pictures of the microchannel sensor design are shown in Figure 1. The vapor sample

flows through the top polycarbonate microchannel and is separated from the liquid

microchannel by a nanoporous membrane. Acetylcholinesterase enzyme is immobilized

in a cross-linked gel at the base of the liquid microchannel. A 40 nm layer of sputtered

gold on the membrane surface acts as the working and counter electrodes for the liquid

microchannel.

The microchannels were machined into the polycarbonate. Then the vapor and liquid

connections were added using 1/16" Teflon tubing. The track-etch polycarbonate

membrane (pore size 10, 100 nm; thickness 10μm; SPI) is sputtered with 40-nm gold.

The membrane electrode coating is used as the working electrode. For some experiments, the working and counter electrodes were both placed on the membrane. In order to create a two electrode system, a shadow mask was used during sputtering. The membrane is then sealed between two polycarbonate microchannels using a thin layer of epoxy.

B. Preparation of Chemicals

Electric eel acetylcholinesterase (EC 3.1.1.7) and the organophosphate, malathion, were purchased from Aldrich. Electric eel acetylcholinesterase is less expensive than human acetylcholinesterase and allows us to use a non-toxic organophosphate. Malathion, commonly used as a pesticide, is not harmful to humans at low exposure levels, but is harmful when used on fish and insects, including electric eels. Malathion vapor was sampled using a bubbler and argon carrying gas, from either the pure liquid or a sample diluted with ethanol. Acetylthiocholine chloride (Sigma) was made to various concentrations in a phosphate buffer. Glutaraldehyde and bovine serum albumin, used to immobilize acetylcholinesterase, were also purchased from Aldrich.

C. Enzyme Immobilization

Acetylcholinesterase was immobilized using the method of Carelli et al for alcohol oxidase immobilization on a gold electrode[27]. The enzyme was cross-linked with bovine serum albumin (BSA) using liquid glutaraldehyde in order to form an immobilized gel: 30 uL of gluteraldehyde was added to 15 uL of 314 U/mL AchE, 8 mg BSA, and 300 uL of phosphate buffer (pH=7.4). The solution (1 uL) is placed on the PDMS microchannel

and allowed to dry for 2 hours. Figure 2 shows QCM data of this cross-linking. The negative delta frequency increases to a sharp peak, the gel point, and then decreases after drying.

D. Optimizing Acetylcholinesterase Chemistry

Acetylcholinesterase chemistry was first optimized in macro-scale experiments. The macro-scale experiments were used to optimize pH, determine degradation temperature, and test enzyme inhibition by malathion. A glassy carbon working electrode, platinum wire counter electrode, and standard Ag/AgCl reference electrode were used (Bioanalytical Systems, Inc). Acetylthiocholine (1 mM) is injected into the enzyme solution (2U/mL in phosphate buffer) at various pH, temperature, and malathion concentrations. The system is incubated for 30 minutes and a cyclic voltammagram (CV) is run from 0.0 to 0.9 V vs Ag/AgCl at a scan rate of 100 mV/sec.

E. Performance testing of the Microchannel Enzyme Sensor

 The acetylthiocholine solution is passed along the liquid microchannel, with or without immobilized acetylcholinesterase, using a syringe pump at 0.01 mL/min. The malathion vapor flows from an argon bubbler and through the gas-phase microchannel at 10 mL/min. A conventional Ag/AgCl electrode (Bioanalytical Systems, Inc) is immersed in a small vial at the outlet of the liquid microchannel. The sensor is held at a constant potential of 800 mV vs. Ag/AgCl and current is measured as the output of the system.

III. Results and Discussion

The effect of pH on acetylthiocholine hydrolysis is shown in Figure 3. For both free and immobilized enzyme the current plateaus above a pH of 7, showing a pH dependence only on the acidic side. At a pH of 6, the current is considerably lower. This decrease in current occurs because of the histidine residue (pKa = 6) in the active site of AChE is only slightly deprotonated at this pH[8].

The degradation temperature of the enzyme was found by performing CVs on acetylcholinesterase solutions in a hot oil bath. Figure 4 shows this decrease occurring between 40 and 45 degrees Celsius with an optimal temperature around 37 degrees Celsius. This data corresponds to previous work documenting acetylcholinesterase behavior in both vertebrates and invertebrates[8].

The results of the initial room temperature inhibition experiments are shown in Figure 5. The first CV curve shows the response of a solution of AChE and acetylthiocholine only. The second CV curve shows the response of a solution of AChE, acetylthiocholine, and malathion. The solution containing malathion shows a 50% decrease in current versus the solution with only acetylthiocholine. This decrease in current is due to the competitive inhibition of the AChE active site due to malathion.

From the macro-scale experiments, it was found that a pH of 7.4 and a temperature of approximately 25 degrees Celsius should be used to test our microchannel sensor. A pH of 7.4 will give a strong current, while working at a temperature sufficiently below the

degradation temperature will enhance enzyme stability. It was also determined, from beaker experiments, that malathion successfully inhibits electric eel acetylcholinesterase and can be used for microsensor testing.

The response of the microchannel sensor to different liquid-phase acetylthiocholine concentrations is shown in Figure 6. As the concentration of acetylthiocholine is increased to 4mM, there is a linear increase in current. Above a concentration of 4mM, the enzyme catalyst becomes saturated and there is negligible current increase. These results are similar to those obtained by previous authors for AChE biosensors[28,29,30,31,32,33]. Due to this similarity, it is assumed the current microchannel sensor set-up accurately portrays the enzyme reaction kinetics.

Four design parameters may affect the response of the sensor to acetylthiocholine and malathion: 1) Location of the counter electrode with respect to the working electrode, 2) the difference in sensor response due to both free and immobilized enzymes, 3) sensor response due to location of the immobilized enzyme, 4) response of the sensor to simulants and interferences.

Amperometric measurements require both a working and counter electrode. When working with such small concentrations, it is often difficult to eliminate IR drop between the working and counter electrodes. The response of the sensor to placement of the counter electrode is shown in Figure 7. There is a higher current response seen when the counter electrode is placed on the nanoporous membrane with the working electrode.

This increase in response indicates that there is a drop in current when the counter electrode is placed at a distance from the working electrode. To eliminate the reduction in current, all further experiments were carried out with the working and counter electrodes on the nanoporous membrane.

The data shown in Figure 7 also indicates that there is little change in sensor response to acetylthiocholine due to immobilization of acetylcholinesterase. Given that the enzyme activities are the same for the free and immobilized acetylcholinesterase, a large difference in sensor response is not expected. When the sensor was exposed to malathion, however, the immobilized enzyme shows a larger percent inhibition than the free enzyme in solution. Percent inhibition is calculated as the current before malathion exposure minus the current after malathion exposure divided by the initial current. The immobilized acetylcholinesterase shows a 33% inhibition when exposed to 52 ppb malathion. Conversely, the acetylcholinesterase in solution was only inhibited 0.6 percent. Comparison data is shown in Figure 8.

Figure 9 demonstrates that the dual microchannel/membrane design can be used as a fast, sensitive sensor. There is a 25 % inhibition of acetylcholinesterase when the sensor is exposed to 0.2 ppb malathion. The response curve contains multiple saturation steps, due to the four active sites of the acetylcholinesterase enzyme. The mass transfer of the gas molecules into the liquid microchannels is efficient; Figure 9 shows that a measurable response occurs in just a few seconds. It takes almost 40 seconds for all four active sites to become saturated. This well exceeds the response time of 10 minutes found by

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previous authors for ppb detection limits of organophosphorus pesticides using acetylcholinesterase[31,34,35].

The sensor is sensitive only to the organophosphorus agents. Table 1 shows the response of the sensor to a variety of organophosphrorus simulants and common interferences. Organic solvents, such as toluene and dodecane, do not produce a response. Also, molecules with similar chemical structures to the organophosphates do not inhibit the acetylcholinesterase sensor. For example, the sensor does not produce a response when exposed to DMMP, which has similar chemical structure to malathion. This selectivity results from using the actual enzyme, thus only those agents which are toxic to the electric eel will show a response. Conventional methods, such as GC/MS and IMS, are not capable of such a high level of selectivity to organophosphates[6]. This selectivity of the sensor is crucial for real-life organophosphorus sensor applications.
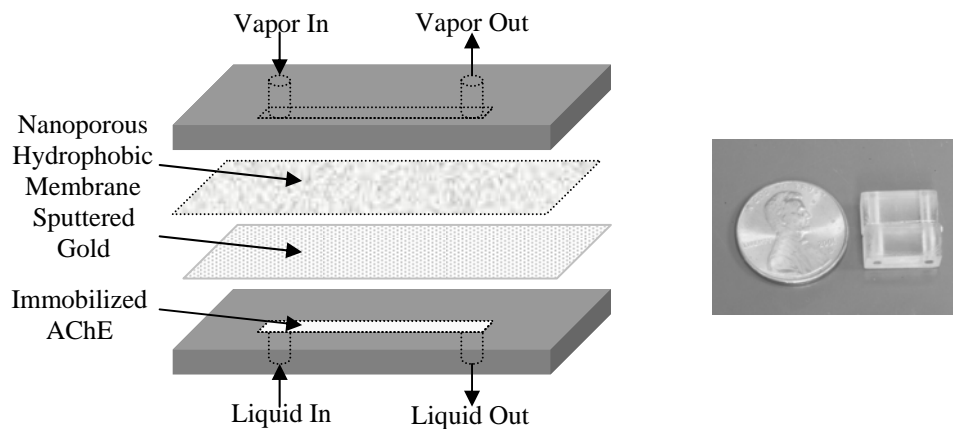
IV. Conclusions

In this study it has been determined that incorporating AChE biochemistry into a microreactor containing a micro-scale gas-liquid interface provides a method to quickly, sensitively, and selectively detect organophosphates in a portable device. This detection is not possible in current GC/MS or IMS techniques.

The use of the dual microchannel/membrane reactor allows for fast diffusion of a concentrated vapor into the liquid microchannel and lowers the detection limit and detection time, compared to previous methods. Using electric eel acetylcholinesterase gives the sensor a higher level of selectivity than previous sensors; only

organophosphates that inhibit the enzyme will give a response. Also, using a microscale

sensor allows the system to be completely portable.

Vapor In   Vapor Out

Nanoporous
Hydrophobic
Membrane
Sputtered
Gold

Immobilized
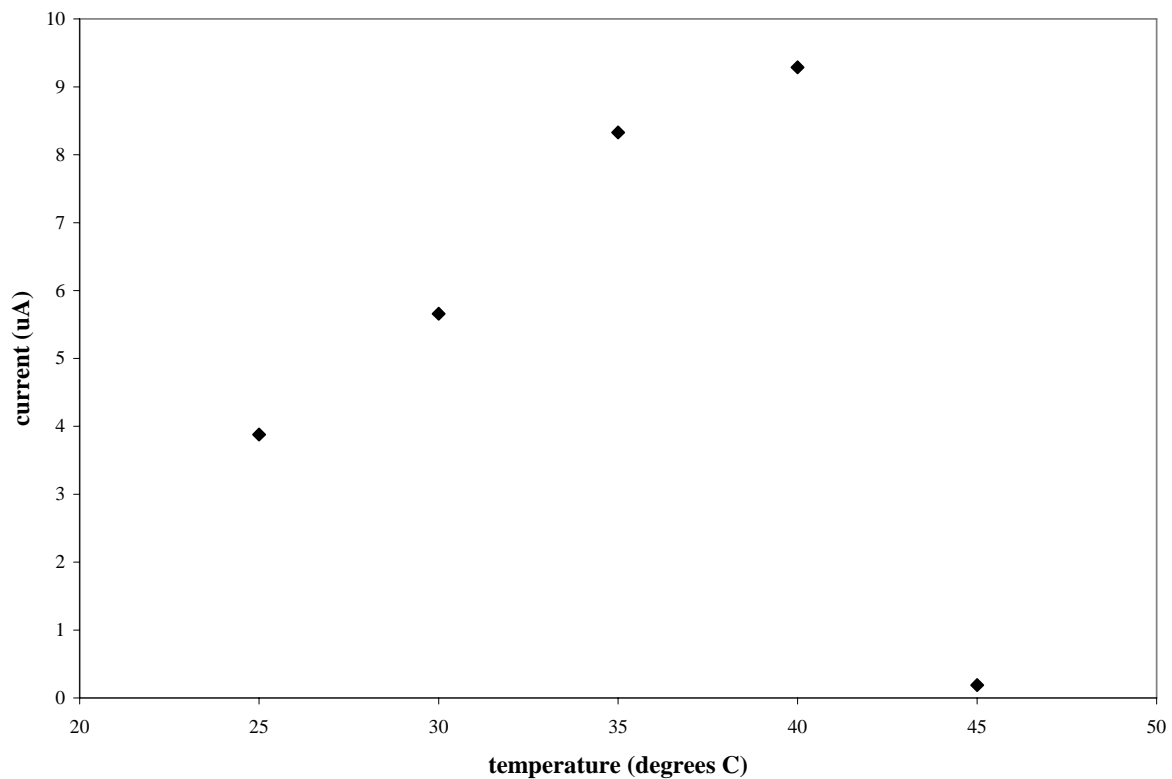AChE

Liquid In   Liquid Out

Figure 1: A) Schematic of the dual microchannel/membrane system. Vapor flows through the top microchannel and is separated from the liquid microchannel by a nanoporous membrane with 40 nm of sputtered gold on the membrane surface closest to the liquid microchannel. B) Picture of polycarbonate microsensor
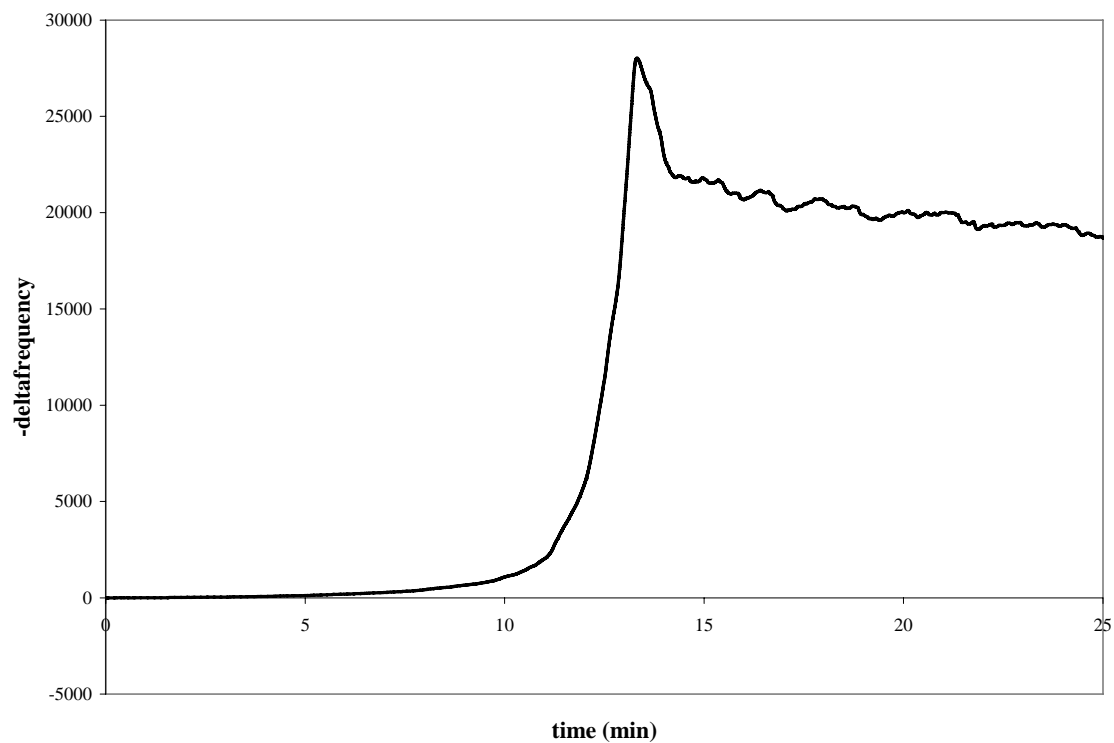
Figure 2: QCM for cross-linking of AChE with BSA. 30 uL of 2.5% glutaraldehyde added to a solution of 15 uL (314 U/mL) AChE, 8 mg BSA, and 300 uL of phosphate buffer (pH = 7.4). The sharp rise in negative delta frequency corresponds to the gel point. A decrease in negative delta frequency corresponds to the drying of the gel.
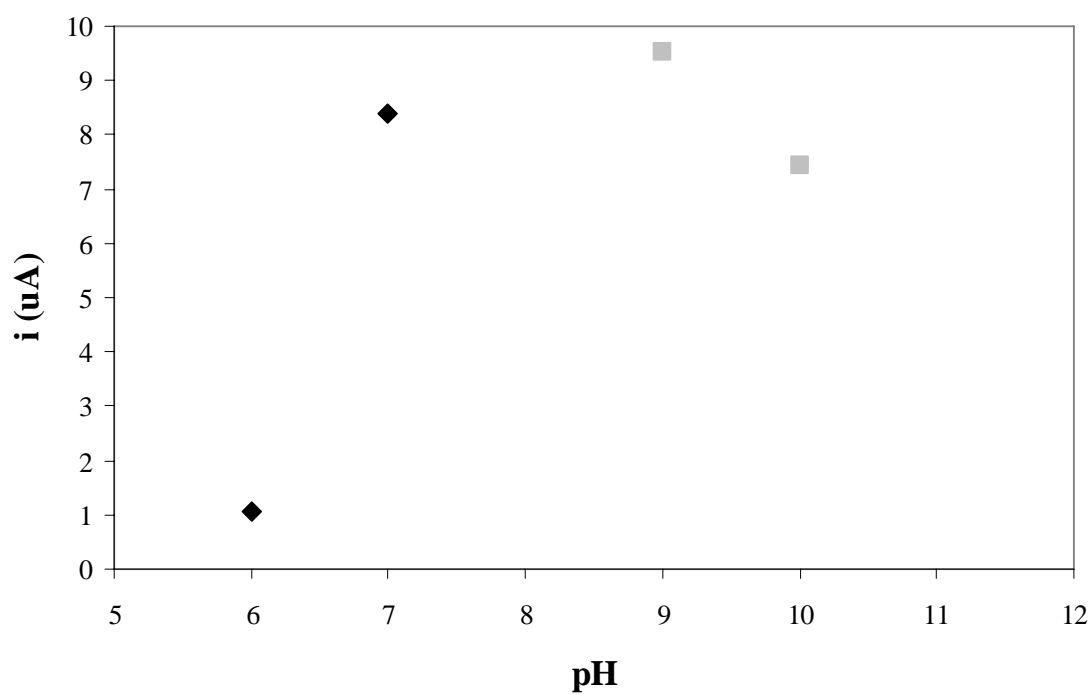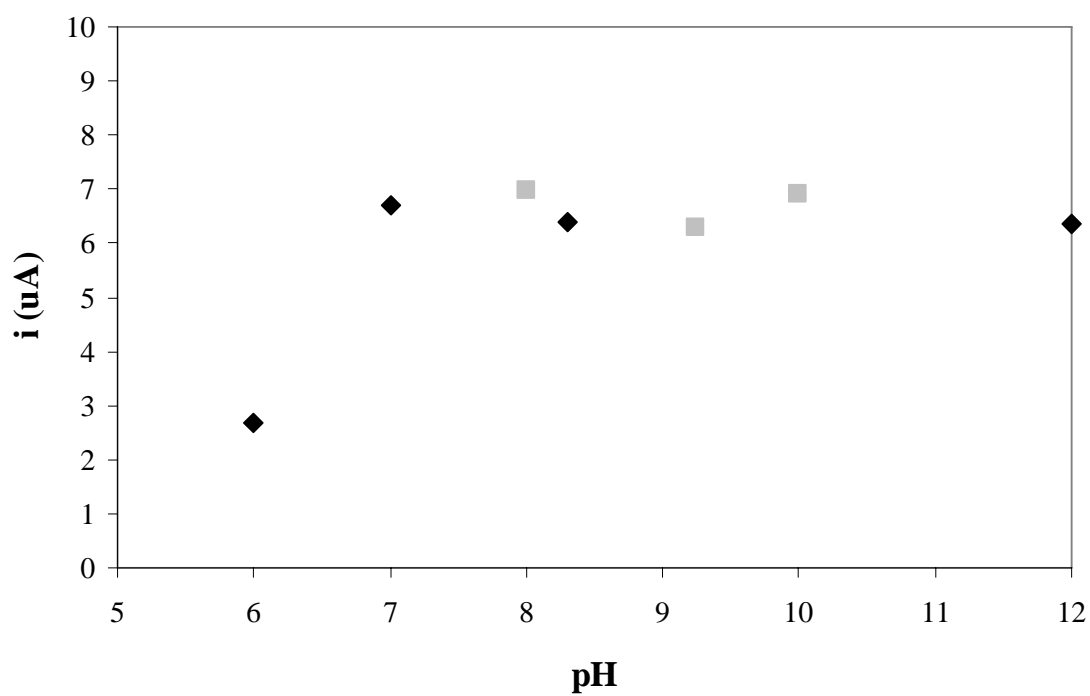
Figure 3: A) Current vs. pH for the hydrolysis of thiocholine from 1 mM acetylthiocholine in a 2 U/mL AChE in phosphate (diamond) and borate (square) buffers at 400 mV vs. Ag/AgCl B) Current vs. pH for the hydrolysis of 1 mM acetylthiocholine in phosphate (diamond) and borate (square) buffer solutions with 0.4 U of AChE

Figure 4: Current vs. temperature for the hydrolysis of thiocholine from 1 mM acetylthiocholine in 2 U/mL AChE in phosphate buffer (pH=7.4). The optimum temperature of the enzyme is approximately 37 degrees Celsius. Enzyme degradation occurs, shown by decrease in current, above 40 degrees Celsius.
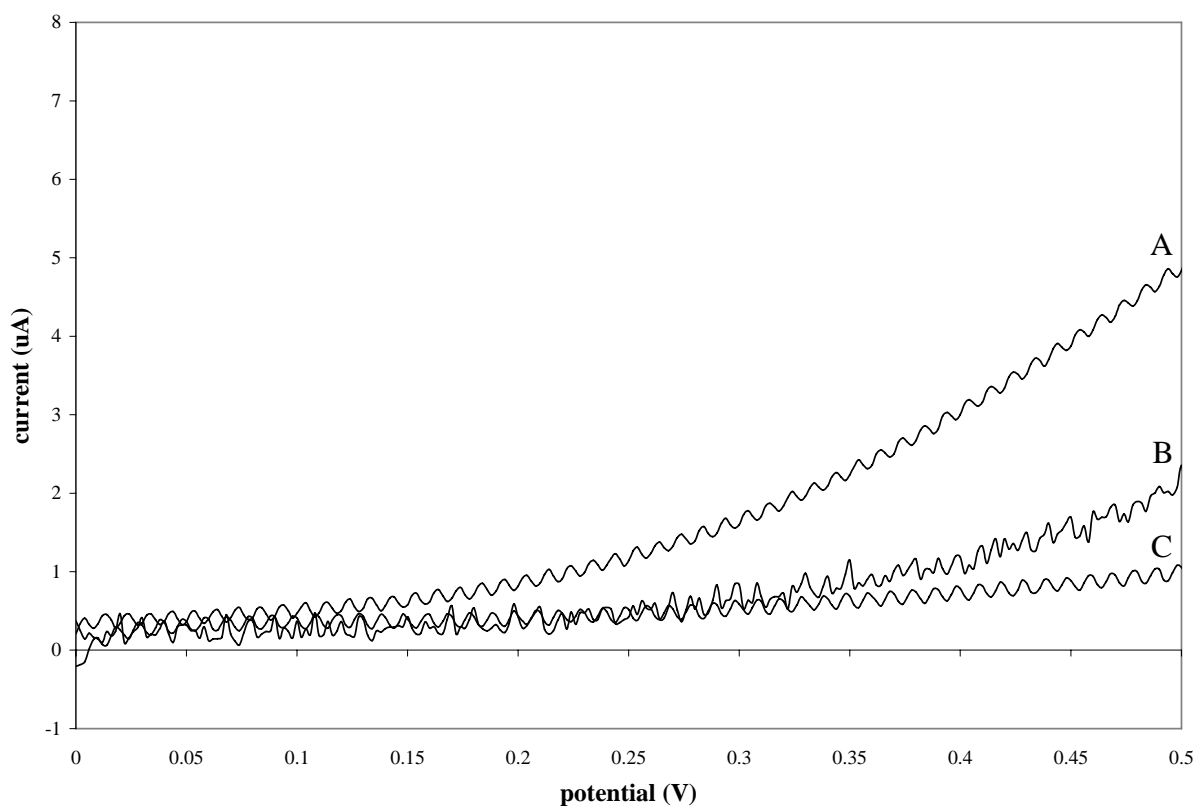
Figure 5: Current vs. potential (vs. Ag/AgCl) for hydrolysis of thiocholine at a scan rate of 100 mV/sec A) 1.69 mM acetylthiocholine + 2.0 U/mL AChE solution, incubated for 30 minutes, B) 1.69 mM acetylthiocholine, 45 uM malathion, 2.0 U/mL AChE solution, incubated for 30 minutes, C) phosphate buffer (pH=7.4)
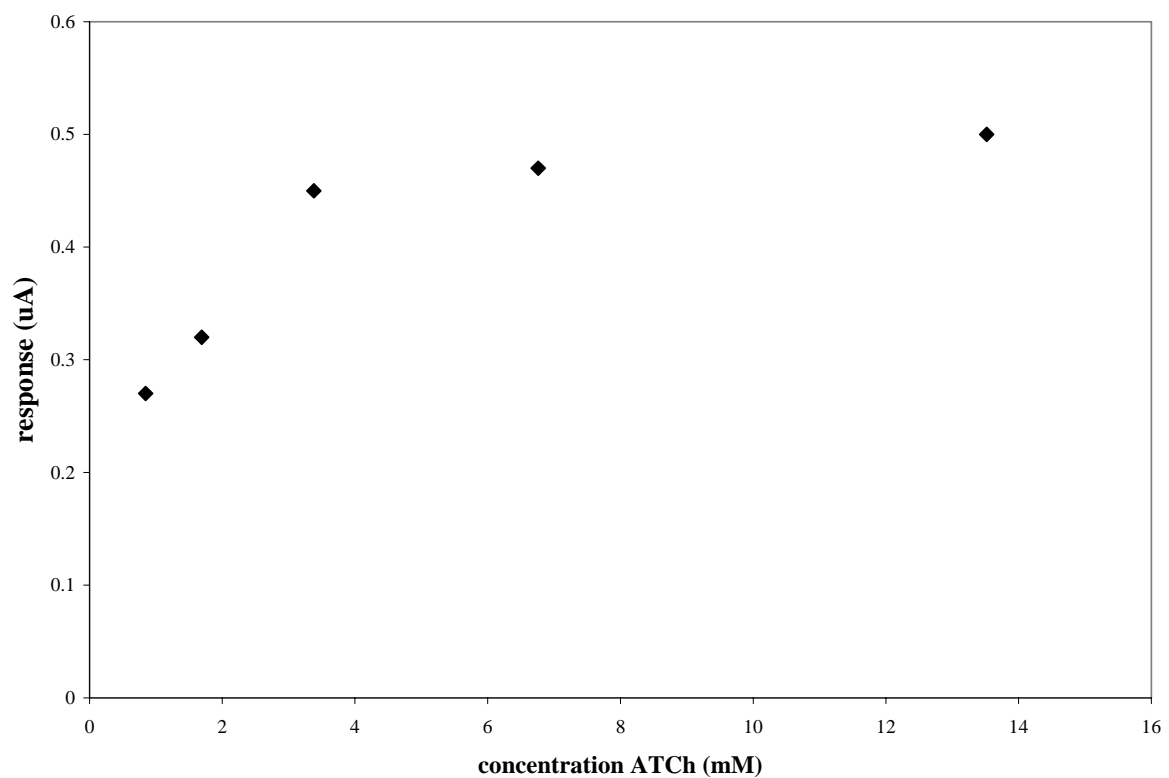
Figure 6: Current vs. acetylthiocholine concentration for 0.01 mL/min of acetylthiocholine flowing over 0.014 U of immobilized AChE
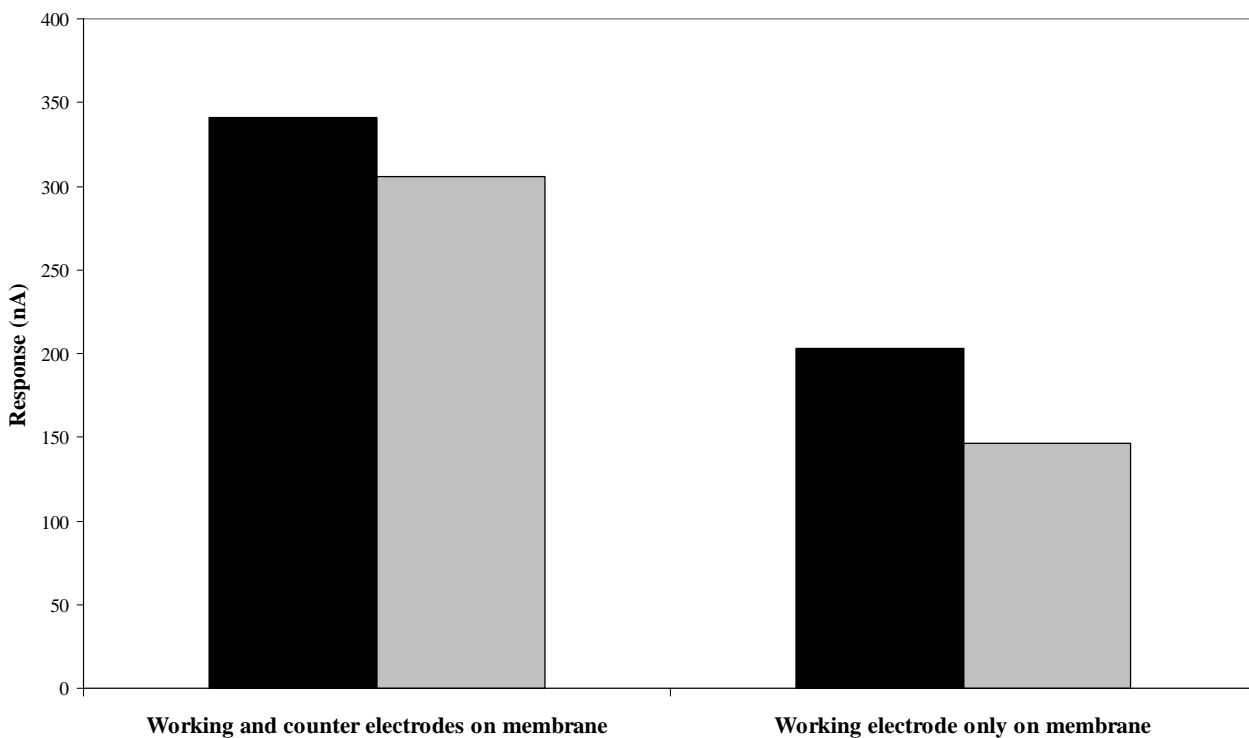
Figure 7: Comparison of sensor response for 1.69 mM acetylthiocholine, 0.013 U AChE, phosphate buffer (pH=7.4) solution with the working electrode only on the nanoporous membrane and the working and counter electrodes in tandem on the membrane. Black bars represent immobilized AChE, while grey bars represent AChE free in solution.
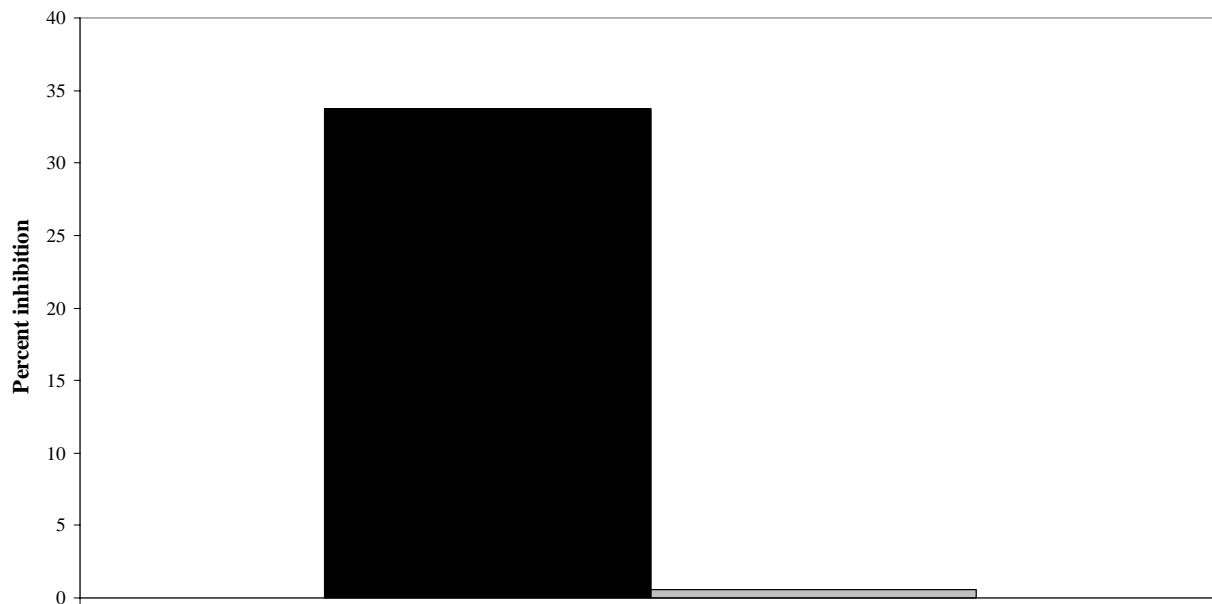
Figure 8: Comparison of percent inhibition after exposure to 52 ppb malathion in argon carrier gas at a rate of 10 mL/min for immobilized AChE (black) and AChE free in solution (grey). Liquid microchannel contains 1.69 mM acetylthiocholine in phosphate buffer solution (pH = 7.4) flowing at
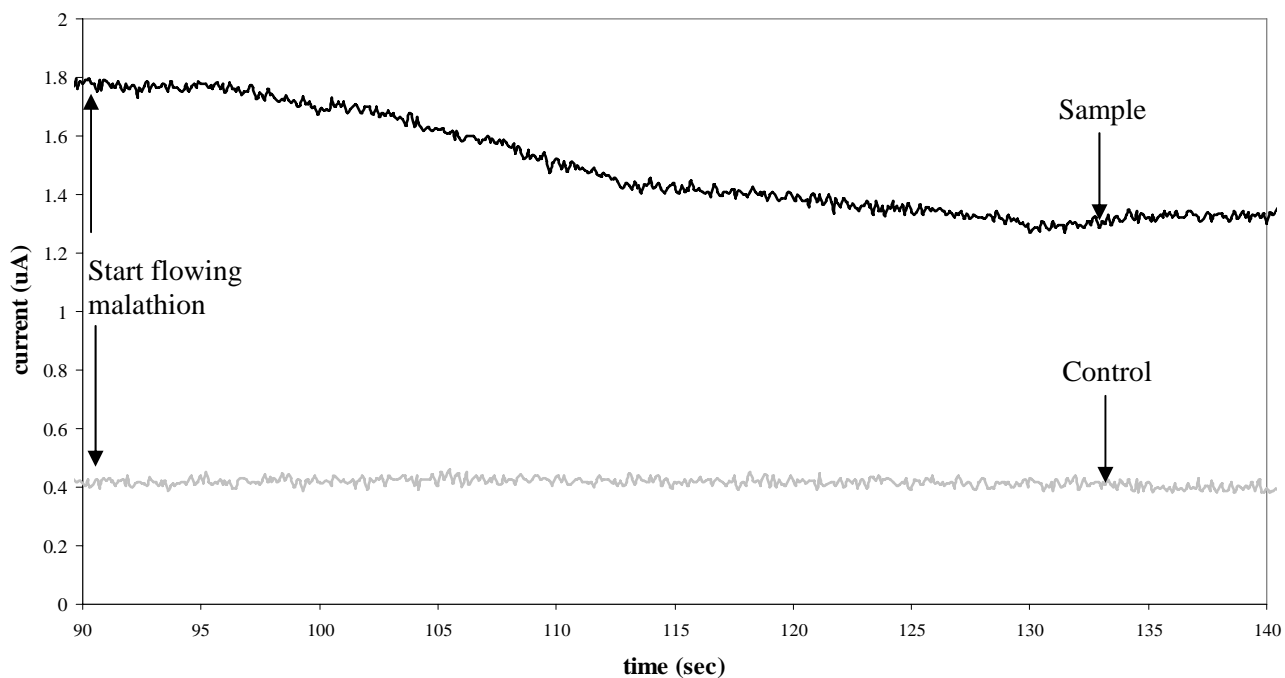
Figure 9: Current vs. time for response of microsensor to 0.2 ppb malathion vapor flowing from bubbler at 10 mL/min for 40 seconds. The liquid microchannel contains 1.69 mM acetylthiocholine in a phosphate buffer solution (pH =7.4) flowing at 0.01 mL/min and 0.013 U of immobilized AChE on bottom of liquid microchannel. The control contains only phosphate buffer (pH = 7.4).

Table 1: Microsensor response to selected simulatants and interferants. The sensor is highly selective and only shows a response when exposed to the organophosphorus AChE inhibitor malathion. The sensor is also highly sensitive and has a detection limit in the parts-per-trillion (ppt).

	Phosphorous Based compound						Other Compounds					
Malathion	DMMP	DEMP	DIMP	DCH	DCP	TEP	Water	Octane	Toluene	Undecane	Dichlorobenzene	Dodecane
ppt	X	X	X	X	X	X	X	X	X	X	X	X

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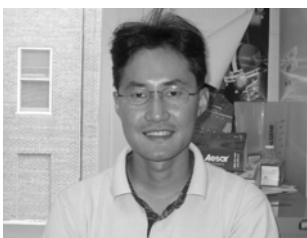
Ilwhan Oh received the PhD in Chemistry in 2002 from Korea Advanced Institute of Science and Technology (KAIST). He worked as a semiconductor research engineer in Samsung Electronics, Inc. Since 2005 he's been working as a post-doctoral research associate in the Department of Chemical and Biomolecular Engineering in the University of Illinois. His research area is micro electrochemical sensors and micro fuel cells.

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VI. References

[1] J. Emsley, *The Shocking History of Phosphorus*. London: Pan Books, 2001

[2] J. Emsley and D. Hall, *The Chemistry of Phosphorus*. New York: John Wiley & Sons, 1976.

[3] J. Akhavan, *The Chemistry of explosives*. Cambridge: The Royal Society of Chemistry,1998.

[4] M. Krausa and A. A. Reznev, "Vapour and Trace Detection of Explosives for Anti-Terrorism Purposes," in *NATO Science Series*, vol. 167. Dordrecht: Kluwer Academic Publishing, 2004.

[5] T. C. Pearce, S. S. Schiffman, H. T. Nagle, and J. W. Gardener, "Handbook of Machine Olfaction: Electronic Nose Technology." Weinheim: Wiley-VCH, 2003.

[6] Y. Sun and K.Y. Ong, *Detection technologies for chemical warfare agents and toxic vapors*, CRC Press, Boca Raton, 2005.

[7] Soreq, *Human Cholinesterases and Anticholinesterases*, Academic Press, London, 1993.

[8] Silver, The *Biology of Cholinesterases, North-Holland*, Amsterdam, 1974.

[9] Liu et al, "Sensitive electrochemical detection of enzymatically generated thiocholine at carbon nanotube modified glassy carobon electrode," Electrochem Comm 7 (2005), 1163-1169.

[10] Pandey et al, "Acetylthiocholin/acetylcholine and thiocholine/choline electrochemical biosensors/sensor based on an organically modified sol-gel glass enzyme reactor and graphite paste electrode," Sens Actuat B 62 (2000), 109-116.

[11] Sotiropoulou et al, "Genetically engineered acetylcholinesterase-based biosensor for attomolar detection of dichlorvos," Biosens Bioelectron 20 (2005), 2347-2352.

[12] Bachmann et al, "Improved multianalyte detection of organophosphates and carbamates with disposable multielectrode biosensors using recombinant mutants of Drosphila acetylcholinesterase and artificial neural networks," Biosens Bioelectron 15 (2000), 193-201.

[13] G. Jeanty and J.L. Marty, "Detection of paraoxon by continuous flow system based enzyme sensor," Biosens Bioelectron 13 (1998), 213-218.

[14] Li et al, "Immobilization of enzyme on screen-printed electrode by exposure to glutaraldehyde vapour for the construction of amperometric acetylcholinesterase electrodes," Anal Chim Acta 382 (1999), 277-282.

[15] A. Gunther and K. F. Jensen, "Multiphase microfluidics: from flow characteristerics to chemical and materials synthesis," *Lab on a Chip*, vol. 6, pp. 1487-1503, 2006.

[16] P. M. Y. Chung and M. Kawaji, "The effect of channel diameter on adiabatic two-phase flow characteristics in microchannels," *International Journal of Multiphase Flow*, vol. 30, pp. 735-761, 2004.

[17] N. de Mas, A. Gunther, T. Kraus, M. A. Schmidt, and K. F. Jensen, "Scaled-out multilayer gas-liquid microreactor with integrated velocimetry sensors," *Industrial & Engineering Chemistry Research*, vol. 44, pp. 8997-9013, 2005.

[18] J. El-Ali, S. Gaudet, A. Gunther, P. K. Sorger, and K. F. Jensen, "Cell stimulus and lysis in a microfluidic device with segmented gas-liquid flow," Analytical Chemistry, vol. 77, pp. 3629-3636, 2005.

[19] A. Gunther, S. A. Khan, M. Thalmann, F. Trachsel, and K. F. Jensen, "Transport and reaction in microscale segmented gas-liquid flow," *Lab on a Chip*, vol. 4, pp. 278-286, 2004.

[20] A. Gunther, M. Jhunjhunwala, M. Thalmann, M. A. Schmidt, and K. F. Jensen, "Micromixing of miscible liquids in segmented gas-liquid flow," *Langmuir*, vol. 21, pp. 1547-1555, 2005.

[21] J. H. Xu, S. W. Li, Y. J. Wang, and G. S. Luo, "Controllable gas-liquid phase flow patterns and monodisperse microbubbles in a microfluidic T-junction device," *Applied Physics Letters*, vol. 88, 2006.

[22] B. K. H. Yen, A. Gunther, M. A. Schmidt, K. F. Jensen, and M. G. Bawendi, "A microfabricated gas-liquid segmented flow reactor for high-temperature synthesis: The case of CdSe quantum dots," *Angewandte Chemie-International Edition*, vol. 44, pp. 5447-5451, 2005.

[23] B. Zheng and R. F. Ismagilov, "A microfluidic approach for screening submicroliter volumes against multiple reagents by using preformed arrays of nanoliter plugs in a three-phase liquid/liquid/gas flow," *Angewandte Chemie-International Edition*, vol. 44, pp. 2520-2523, 2005.

[24] J. Kobayashi, Y. Mori, K. Okamoto, R. Akiyama, M. Ueno, T. Kitamori, and S. Kobayashi, "A microfluidic device for conducting gas-liquid-solid hydrogenation reactions," *Science*, vol. 304, pp. 1305-1308, 2004

[25] B. Zhao, J. S. Moore, and D. J. Beebe, "Surface-directed liquid flow inside microchannels," *Science*, vol. 291, pp. 1023-1026, 2001.

[26] B. Zhao, J. S. Moore, and D. J. Beebe, "Principles of surface-directed liquid flow in microfluidic channels," *Analytical Chemistry*, vol. 74, pp. 4259-4268, 2002.

[27] Carelli et al, " An interference-free first generation alcohol biosensor based on a gold electrode modified by an overoxidised non-conducting polypyrrole film," Anal Chim Acta 565 (2006), 27-35.

[28] C. Chouteau et al, "A bi-enzymatic whole cell conductometric biosensor for heavy metal ions and pesticides detection in water samples," Biosens Bioelectron 21 (2005), 273-281.

[29] H. Shi et al, "Fabricating of Acetylcholine Biosensor by a Layer-by-layer Deposition Technique for Determing Trichlorfon," Electroanal 17 (2005), 1285-1290.

[30] L. Doretti et al, "Acetylcholine biosensor involving entrapment of acetylcholinesterase and poly(ethylene glycol)-modified choline oxidase in a poly(vinyl alcohol) cryogel membrane," Enzyme and Microbial Technology 27 (2000), 279-285.

[31] S. Dzyadevych et al, "Early-warning electrochemical biosensor system for environmental monitoring based on enzyme inhibition," Sensors and Actuators B 105 (2000), 81-87.

[32] M. Yang et al, "Microbiosensor for acetylcholine and choline based on electropolymerization/sol-gel derived composite membrane," Anal Chim Acta 530 (2005), 205-211.

[33] A.M. Hendji et al, "Sensitive detection of pesticides using a differential ISFET-based system with immobilized cholinesterase," Anal Chim Acta 281 (1993) 3-11.

[34] R.E. Gyurcsanyi et al, "Fast Response Potentiometric Acetylcholine Biosensor," Electroanal 11 (1999) 712-718.

[35] E. Suprum et al, "Acetylcholinesterase sensor based on screen-printed carbon electrode modified with prussian blue," Anal Bioanal Chem 383 (2005), 597-604.